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(54) Title: COMPOSITIONS FOR THE TREATMENT OF WOUND CONTRACTURE

(57) Abstract: The present invention provides the use of an oxidized cellulose for the preparation of a medicament for use in the treatment or prevention of wound contracture. Preferably, the oxidized cellulose is oxidized regenerated cellulose (ORC) or partially hydrolysed ORC.

COMPOSITIONS FOR THE TREATMENT OF WOUND CONTRACTURE

The present invention relates to the use of an oxidized cellulose for the preparation of medicaments for use in the treatment or prevention of wound contracture, in particular the prevention of burn contracture.

Wound contracture is the process which diminishes the size of a full-thickness open wound, especially a full-thickness burn. The tensions developed during contracture and the formation of subcutaneous fibrous tissue can result in deformity, and in particular to fixed flexure or fixed extension of a joint where the wound involves an area over the joint. Such complications are especially prevalent in burn healing.

Fibroblasts have been implicated in the mechanism of wound contraction.

Accordingly, it has been suggested to use substances or procedures which interfere with myofibroblast mobilisation, migration, adhesion or multiplication for the inhibition of wound contracture. For example, high doses of cortisone or related steroids have been shown to suppress fibroblast proliferation, and thereby inhibit wound contracture. Unfortunately, steroids given in such high doses give unacceptable side effects in clinical practice.

Cellular poisons such as cyanide and dinitrophenol, have also been reported to inhibit wound contraction. Likewise, drugs which inhibit smooth muscle contraction have been reported to inhibit wound contraction, for example, colchicine, vinblastine and phenyltoin.

It has been found that an adherent dressing, such as untreated gauze, can delay, but does not prevent contracture. In contrast, certain synthetic films such as nylon applied to the wound surface before active contracture has started can inhibit contracture.

It has also been found that application of a full-thickness skin graft to an open wound before wound contracture commences is effective to prevent wound contracture. However, significant problems are associated with skin grafting including cost, the source of grafting skin, rejection of the graft, secondary infection, and associated surgical risks.

US-A-4957902 describes the inhibition of wound contracture by application to the wound of polypeptides having an amino acid sequence which is similar or identical to certain amino acid sequences of type I collagen.

It has now been found that certain oxidized cellulose derivatives display promising activity *in vitro* that is indicative of ability to prevent or reduce wound contracture.

Accordingly, the present invention provides the use of an oxidized cellulose for the preparation of a medicament for use in the treatment or prevention of wound contracture.

The term "oxidized cellulose" refers to any material produced by the oxidation of cellulose, for example with dinitrogen tetroxide. Such oxidation converts primary alcohol groups on the saccharide residues to carboxylic acid groups, forming uronic acid residues within the cellulose chain. The oxidation generally does not proceed with complete selectivity, and as a result hydroxyl groups on carbons 2 and 3 are occasionally converted to the keto form. These keto units introduce an alkali label link, which at pH 7 or higher initiates the decomposition of the polymer via formation of a lactone and sugar ring cleavage. As a result, oxidized cellulose is biodegradable and bioabsorbable under phsyiological conditions.

The preferred oxidized cellulose for practical applications is oxidized regenerated cellulose (ORC) prepared by oxidation of a regenerated cellulose, such as rayon. It has been known for some time that ORC has haemostatic properties. ORC has been available as a haemostatic product called SURGICEL

(Registered Trade Mark of Johnson & Johnson Medical, Inc.) since 1950. This product is produced by the oxidation of a knitted rayon material.

A modification of porosity, density and knit pattern led to the launch of a second ORC fabric product, INTERCEED (Registered Trade Mark of Johnson & Johnson Medical, Inc.), which was shown to reduce the extent of post-surgical adhesions in abdominal surgery.

WO98/00180 describes the use of ORC and complexes thereof for the treatment of chronic wounds, such as diabetic ulcers. The mechanism of action of the ORC in chronic wound treatment is thought to involve binding and inactivation of matrix metalloproteinase enzymes present in the wound fluid.

WO98/00446 describes the preparation of ORC oligosaccharides by partial hydrolysis of ORC in alkali solution, followed by dialysis and purification. The ORC oligosaccharides are shown to have similar matrix metalloproteinase binding properties to ORC, and are also indicated for the treatment of chronic wounds.

Accordingly, in the use according to the present invention, the oxidized cellulose preferably comprises oxidized regenerated cellulose, and more preferably the oxidized cellulose is partially hydrolysed. More preferably, the partially hydrolysed oxidized cellulose is water soluble at 25°C to an extent of at least 1g/l. More preferably, the partially hydrolysed oxidized cellulose has a weight average molecular weight in range of 1000 to 50,000.

It has been found by means of the *in vitro* fibroblast populated collagen gel contraction model described in detail below under procedure 1 that such partially hydrolysed ORC is surprisingly effective to inhibit collagen gel contraction. This effect was observed with both neonatal and foetal fibroblasts. It was further found that the ORC fragments inhibited the stimulatory effect of certain growth factors on fibroblast populated collagen gel contraction. These results provide a clear indication that the ORC fragments are likely to be effective to reduce wound contracture *in vivo*. Furthermore, these results indicate that ORC itself when used

as a dressing is likely to reduce wound contracture *in vivo* since the ORC breaks down gradually in the wound bed to ORC fragments.

Preferably, in the use according to the present invention, the medicament comprises a wound dressing. For example, the oxidized cellulose may be in form of a woven or nonwoven fabric that is applied to the surface of the wound to prevent contracture.

In preferred embodiments of the present invention, the oxidized cellulose is complexed with collagen to form structures of the kind described in WO98/00180 and WO98/00446, the entire contents of which are expressly incorporated herein by reference. For example, the oxidized cellulose may be in the form of milled ORC fibres that are dispersed in a freeze-dried collagen sponge. This provides for sustained release of the oxidized cellulose to the wound, together with certain therapeutic and synergistic effects arising from the complexation with collagen.

In other preferred embodiments, the use according to the present invention provides a medicament in the form of an ointment gel or film or sponge for application to a wound.

The medicaments provided by the present invention are preferably applied to a wound or a burn shortly after the wound or burn has been inflicted. Preferably, the application is continued until the epitheliasation of the wound is complete. Preferably, the wound is a full-thickness wound or burn. Preferably, the wound is a burn. Preferably, the area of the wound is at least $5cm^2$, more preferably at least $10cm^2$, and most preferably at least $20cm^2$.

Accordingly, in a further aspect, the present invention provides a method for inhibiting wound contraction comprising the steps of:

(a) providing a pharmaceutically acceptable composition including as the active principle an oxidized cellulose; and

(b) administering a therapeutically effective amount of the pharmaceutically acceptable composition to an individual in need thereof.

It will be appreciated that the present application can also be useful in the treatment of internal wounds. Administration may be by any means that facilitate the contracture-inhibiting effect of the oxidized cellulose. Preferably, the administration is topical.

Particular embodiments of the present invention will now be described further, by way of example, with reference to the accompanying drawings, in which:-

Figure 1 illustrates the effect of partially hydrolysed oxidized cellulose on foetal fibroblast populated collagen gel fibroblast contraction, including data for measurements carried out in the presence of selected growth factors, (a) at time T=0 and (b) T=15 days; and

Figure 2 shows similar data to those in Figure 1, the only difference being that the test is carried out for a neonatal fibroblast loaded collagen gel.

Procedure 1

The inhibitory effect of selected compounds on fibroblast populated collagen gel contraction was determined by a method similar to that described in US-A-4957902.

Briefly, fibroblast populated collagen gels were prepared as follows: neonatal and foetal fibroblasts (HSF 43 SK and FF 1475) were grown to confluency in 10% Fetal Bovine Serum (FBS)/Dulbecco's Modified Eagles Medium (DMEM). The cells were harvested using 0.05% trypsin/EDTA, counted and centrifuged to remove trypsin solution. The cells were resuspended at a cell

density of 140,000 cells/ml, which is four times the required cell density in the collagen gel.

The following mixture was then made up: 14mls 10% FBS/DMEM, 7mls of the cell suspension, and 7mls of rat tail collagen type I (final concentration 1mg/ml). The mixture was then distributed at 1ml/well in a 24-well plate and allowed to gel at 37°C for 1hr.

Once the gels had polymerised they were rimmed with a sterile pipette tip, and then additional 0.5ml aliquots of 1% FBS/DMEM were added carefully to each well. The additional medium contained the ORC and/or growth factor of choice. Growth factor concentrations were as follows: TGFß1, final concentration 2.5ng/ml; PDGF-BB, final concentration 10ng/ml and ß-FGF, final concentration 3ng/ml. When present, the ORC was a soluble fragment prepared in accordance with Example 1 hereinbelow at a concentration of 0.5mg/ml, giving a final concentration in the gels of 0.165mg/ml. All solutions were sterile filtered. Each growth factor was incubated for 1hr at 37°C in the stock solution before its addition to the rimmed collagen gels.

Contraction of the collagen gels was measured by taking photographs of each gel over 15 days, and measuring the gel area from the photographs. The results can be summarised as follows, with reference to the Figures.

Referring to Figure 1(a), this bar chart shows the collagen gel area at t=1 day for the collagen gels populated with fetal fibroblasts. The samples with ORC present are shown as hatched bars. The control samples are shown as unhatched bars. Data are shown for collagen gels containing: no added growth factors, added TGF-b1, PDGF-BB, and b-FGF. It can be seen that the gel areas are in the range 45-70 mm^2, and that there is no statistically significant difference between the areas of the test gels and control gels.

Referring to Fig. 1(b), this shows the areas of the gel samples of Fig. 1(a) at t=15 days. It can be seen that all of the gels have shrunk very significantly due to

fibroblast proliferation in the gel, with the resulting gel areas all falling in the range 8-16 mm^2. However, it can also clearly be seen that the samples containing ORC oligosaccharides exhibited significantly less contracture of the gel area after 15 days than the samples that were free from hydrolysed ORC. The same effect is observed for the samples containing TGF-β1, PDGF-BB and β-FGF, indicating that the hydrolysed ORC is effective to significantly reduce contracture relative to the control even in the presence of these growth factors.

Figure 2 shows data obtained and displayed in identical fashion to the data of Fig. 1, but using neonatal fibroblast populated collagen gels instead of the foetal fibroblast populated collagen gels of Fig. 1. It can be seen that the measured contracture of the collagen gels is generally greater for the neonatal fibroblast populated gels of Fig. 2 than for the foetal fibroblast populated gels of Fig. 1. This reflects the inherently lower tendency of foetal wounds to form scars. However, the significant reduction of gel contracture in the presence of ORC is also apparent for the neonatal fibroblast populated gels of Fig. 2.

Example 1

Soluble hydrolysed ORC was prepared as described in WO98/00446. Briefly, a SURGICEL ORC fabric was dissolved in 6M sodium hydroxide at a concentration of 20mg/ml. The solution was incubated at 37°C for 45 minutes, after which the reaction was stopped by adding 5M HCl until precipitation occurred and the pH changed from alkaline to pH7 of less. The precipitate was allowed to settle overnight, and then the excess liquid was removed. The precipitate was dialysed against water in tubing with a 1000 molecular weight cut off, then freeze dried to produce a powder.

The molecular size of the oligosaccharide, determined by gel electrophoresis and by high performance liquid chromatography, showed a range extending from approximately 1000 to 15000 daltons. The oligosaccharide is soluble in water at pH7 and above.

Example 2

A collagen/ORC sponge dressing suitable for application to a large-area burn to reduce contracture is prepared as described in WO98/00180. Briefly, the freeze-dried collagen prepared as described in US-A-4614794 or US-A-4320201 is re-suspended in 0.05m acetic acid at a concentration of 10mm/M. Milled ORC powder (milled SURGICEL cloth) is added to the suspension at a ratio of 1:3 ORC:collagen and homogenized using a Waring blender on low speed for 3x30 seconds. The complex suspension is degassed in a vacuum oven for 10 minutes, and is then poured to a depth of 3mm into a tray and blast frozen. The frozen suspension is then either freeze-dried and dehydrothermally cross-linked using a programmable freeze-drier with a temperature ramping facility, or it is dried using a solvent drying process as described in US-A-2157524.

Example 3

A gel ointment suitable for application to wounds for the prevention of contracture is prepared according to the following formulation:-

Carboxymethylcellulose	2.4%
Hydroxyethylcellulose	0.3%
Sodium chloride	0.24%
Propylene glycol	20.2%
Hydrolysed ORC (from Example 1)	2%
Water	balance

The sterile pharmaceutical gel was formulated under aseptic conditions.

The above embodiments have been described by way of example only. Many other embodiments falling within the scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. Use of an oxidized cellulose for the preparation of a medicament for use in the treatment or prevention of wound contracture.

2. Use according to claim 1, wherein the oxidized cellulose comprises oxidized regenerated cellulose.

3. Use according to claim 1 or claim 2, wherein the oxidized cellulose is partially hydrolysed.

4. Use according to claim 3, wherein the partially hydrolysed oxidized cellulose has a molecular weight in the range of 1000 to 50,000.

5. Use according to any preceding claim, wherein the medicament comprises a wound dressing.

6. Use according to claim 5, wherein said oxidized cellulose is in the form of a woven or nonwoven fabric.

7. Use according to any preceding claim, wherein said medicament comprises said oxidized cellulose complexed to collagen.

8. Use according to any preceding claim, wherein said wound contracture comprises burn contracture.

9. Use according to any preceding claim, wherein said medicament comprises an ointment for application to a wound.

FIG. 1(a)

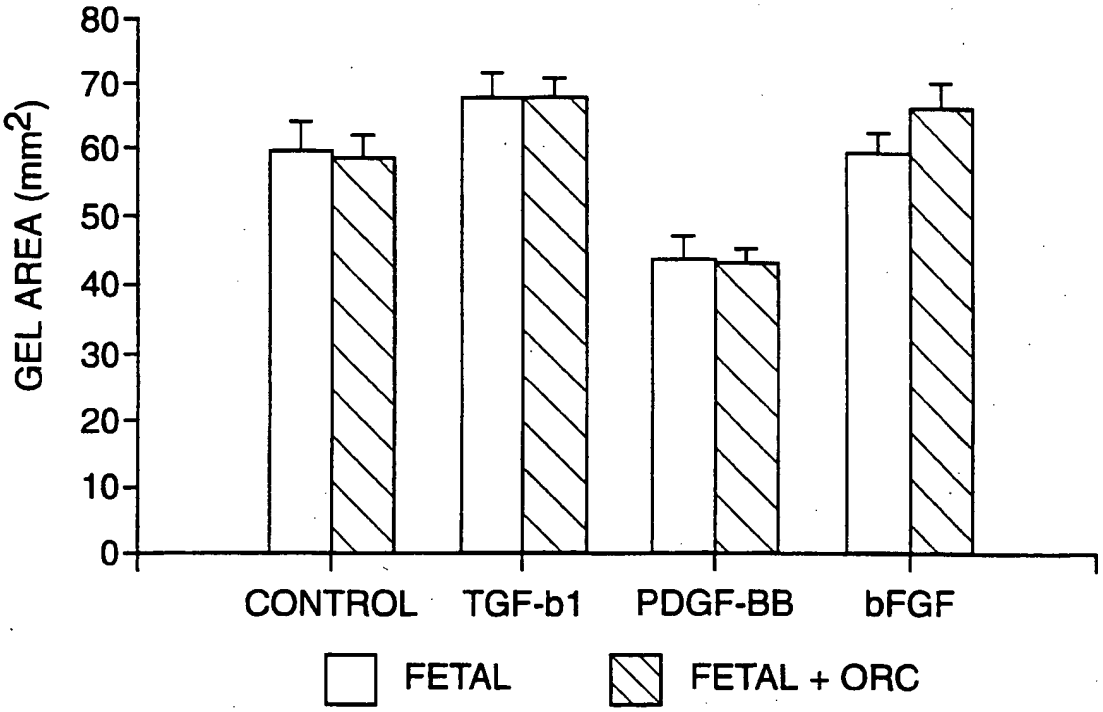

GEL AREA (mm2)
80
70
60
50
40
30
20
10
0
CONTROL
TGF-b1
PDGF-BB
bFGF
FETAL
FETAL + ORC

FIG. 1(b)

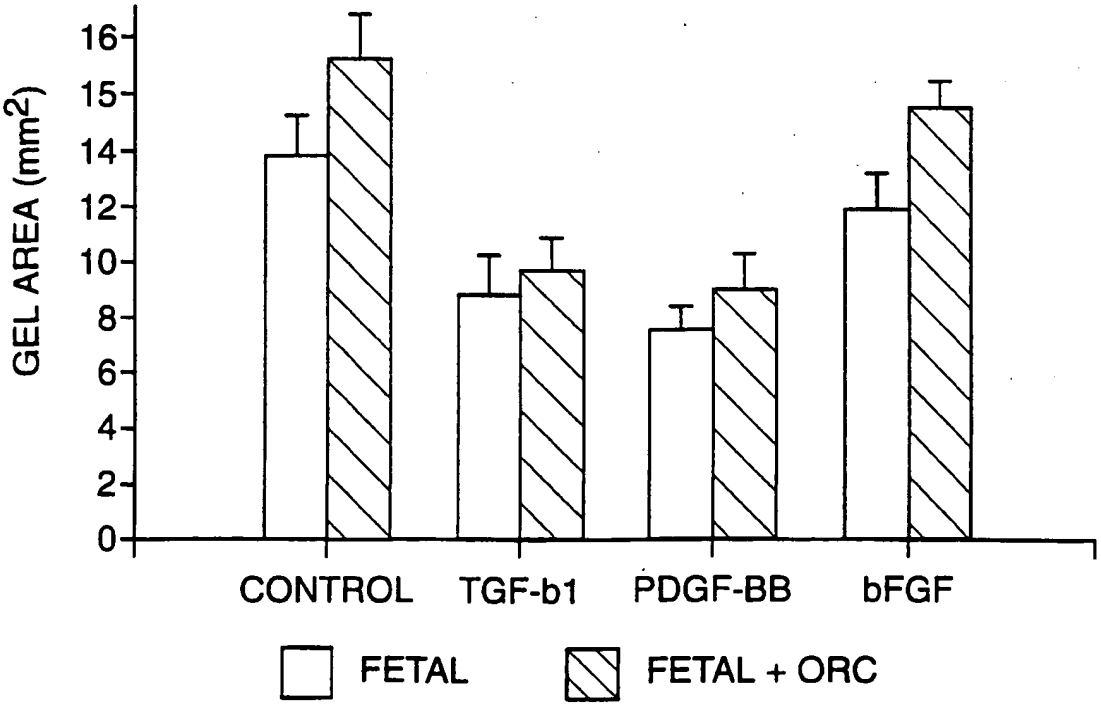

GEL AREA (mm2)
16
15
14
12
10
8
6
4
2
0
CONTROL
TGF-b1
PDGF-BB
bFGF
FETAL
FETAL + ORC

FIG. 2(a)

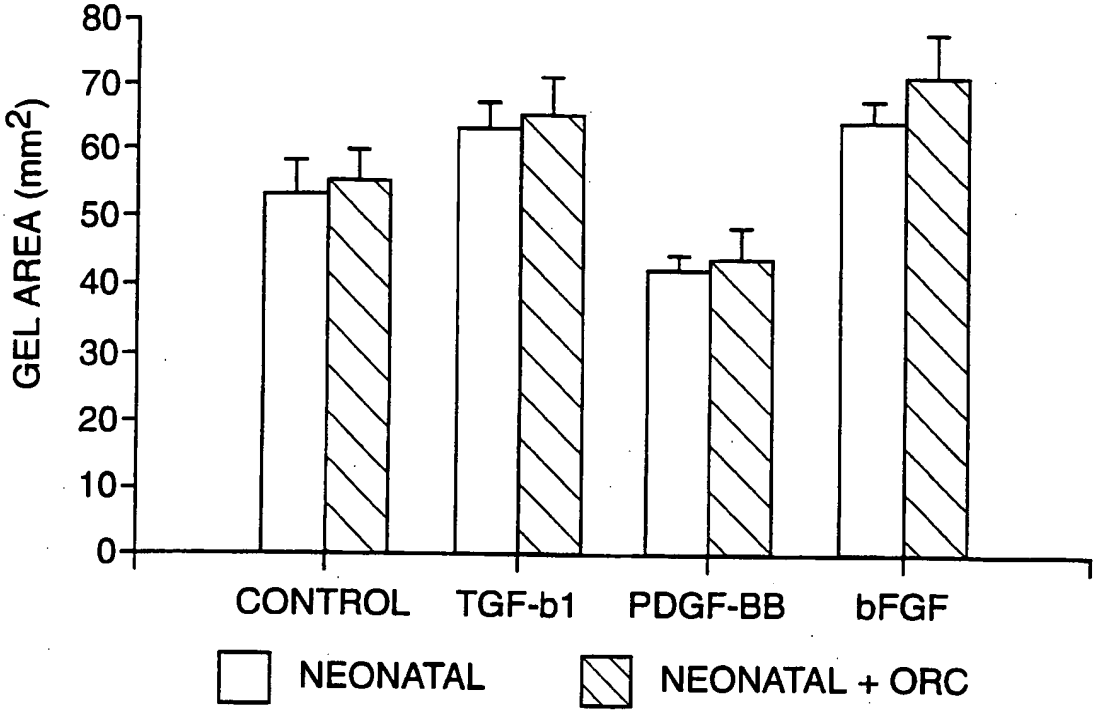
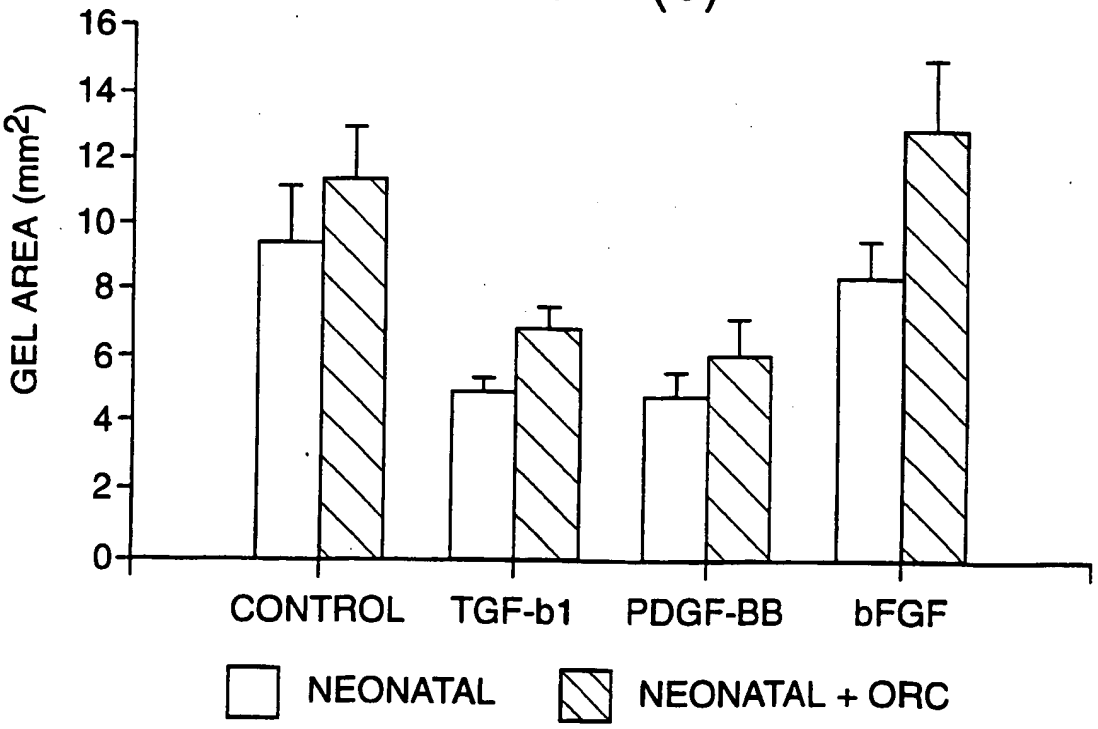

GEL AREA (mm2)
80
70
60
50
40
30
20
10
0
CONTROL
TGF-b1
PDGF-BB
bFGF
NEONATAL
NEONATAL + ORC

FIG. 2(b)


GEL AREA (mm2)
16
14
12
10
8
6
4
2
0
CONTROL
TGF-b1
PDGF-BB
bFGF
NEONATAL
NEONATAL + ORC

INTERNATIONAL SEARCH REPORT

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PCT/[redacted]0/03744

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L15/28 A61L15/22 A61L26/00 A61K47/38

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 696 101 A (HOPKINS WARREN KENT ET AL) 9 December 1997 (1997-12-09) column 3, line 38 - line 43; claims ---	1-9
X	GB 2 314 840 A (JOHNSON & JOHNSON MEDICAL) 14 January 1998 (1998-01-14) claims; examples & WO 98 00446 A 8 January 1998 (1998-01-08) cited in the application ---	1-9
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Interr [redacted]al Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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